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PREPARATION AND CHARACTERIZATION OF PARTICULATE ENVIRONMENTAL CONTAMINANTS FOR BIOLOGICAL EXPERIMENTS

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16. ABSTRACT

The purpose of this project was to provide a centralized source for the preparation and characterization of selected particulate materials for biological experiments. The particulate materials of interest were a range of environmental contaminants known or suspected to detrimentally affect human health. The prepared materials were delivered to personnel at the Environmental Protection Agency and to designated EPA contractors.

The centralized source approach helped to assure that the results of biological experiments conducted at different laboratories were comparable. In addition, the centralized source approach placed specialists in particle technology at the disposal of biological researchers. Thus, more intricate particle preparation procedures could be undertaken than would otherwise be the case.

Source powders were collected, size classified, and characterized for use. Preparations were shipped to (1) State University of New York at Stony Brook for two long-term series of experiments, (2) life sciences Division of IIT Research Institute for two series of experiments, and (3) EPA/Cincinnati.

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PREPARATION AND CHARACTERIZATION OF FINELY DIVIDED PARTICULATE ENVIRONMENTAL CONTAMINANTS FOR BIOLOGICAL EXPERIMENTS

by

George Yamate and Hubert Ashley
IIT Research Institute
Chicago, Illinois 60616

Contract No. 68-02-1687

Project Officer

David Coffin
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, N.C. 27711

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
HEALTH EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, N.C. 27711

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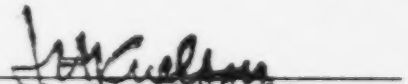
FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The purpose of this project was to provide a centralized source for the preparation and characterization of selected particulate materials for biological experiments. The particulate materials of interest were a range of environmental contaminants known or suspected to detrimentally affect human health.



John H. Knelson, M.D.
Director,
Health Effects Research Laboratory

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SECTION 1

INTRODUCTION

The objective of this project was to obtain a variety of high purity particulate substances whose size distribution was reliably and carefully characterized, and when necessary reduced and/or selectively fractionated into a size range of interest for various biological experiments. The experimental protocols employ particulate suspensions for intratracheal instillations in small animals, for inhalation exposures of animals to aerosols of these substances, and for administration of particulates to *in vitro* cell culture systems. In addition, a limited number of different preparations of selected, finely divided particulate metal or metal oxides in combination with the polycyclic aromatic hydrocarbon carcinogen, benzo(a)pyrene, (BP) or other designated carrier material were required for experimental use. These various preparations were to be used by several different investigators supported by EPA contracts, as well as in-house investigators at the National Experimental Research Center, Research Triangle Park, North Carolina 27711.

The project was designed to provide a centralized, consistent source for particle characterization and preparation. This would eliminate one potential cause of factitious variability between different investigators seeking to contrast biological consequences of environmental contaminants. Assuming a uniform, highly reproducible particle preparation for all related investigations will help to provide meaningful data on the basis of which observed differences in test effects can be better understood. In the absence of such assurance, apparent differences may be due to or masked by variations in the physical parameters as well as accession or retention in biologically significant sites.

Compounds from the following list were to be characterized, prepared, and supplied as required by the Project Officer. These were:

Platinum Sulfate - $Pt(SO_4)_2$

Platinum Metal

*Platinum Oxide - PtO_2

Palladium Metal

Palladium Oxide - PdO

*Lead Oxide - PbO

*Iron Oxide - Fe_2O_3

Cadmium Oxide

Nickel Oxide - NiO

Vanadium - V_2O_5, V_2O_3

Manganese - MnO, MnO_2

Chromium Oxide - Cr_2O_3

*Crude Air Particulate (particle samples collected for EPA by electrostatic precipitation from air of a large industrial city)

Each powdered compound was to be prepared approximately in the 0.5-5.0 μm range.

Sterile, stable preparations of the four compounds designated above with an asterisk were to be prepared in combination with benzo(a)pyrene (BP) for intratracheal instillation. Precipitation as well as grinding methodology were used to prepare these combination preparations. The powdered compounds both with and without BP were prepared according to the needs and requirements of the Project Officer, and on a time schedule satisfactory to the contractors experimental program.

SECTION 2

DESCRIPTION OF WORK EFFORT

PARTICULATE SUBSTANCES OF INTEREST

The chemicals of interest to the program were obtained from chemical supply houses and the EPA. The approximate data on the powders were obtained by physical and chemical analysis as well as from information supplied by the manufacturer. An initial list of sources and data obtained is given in Table 1.

Classification of Source Powders

Since the powders in Table 1 contained the desired range of particle size (0.5-5.0 μm), a cascade sedimentation method was selected to obtain the size distribution (fraction) of interest. In an earlier study of a similar nature, the cascade sedimentation method was found to be highly successful in obtaining a selected size fraction. In addition, the method is a classification technique based on Stoke's diameter (aerodynamic size) which more closely resembles the diameter of interest in inhalation studies. Particles were thus separated and sized according to their Stoke's diameter. In this context, Stoke's diameter is defined as "the diameter of the sphere with the same terminal settling velocity as the particle". Thus, under conditions of any degree of shape variation, microscope "size" and Stoke's diameter are not related.

Cascade Sedimentation Method

The Cascade Sedimentation Method is based on Stoke's equation

$$d^2 = \frac{18\eta\, h \times 10^8}{(\rho_s - \rho_f)gt}$$

TABLE 1. SOURCES OF RAW POWDERS

Powder	Supplier	Cost	Approximate Characteristics
Platinum Oxide	Platinum Chemical New Jersey	$12/g	PSR 1/2-20 μm; D = 14.9; P = 99.9
Palladium Oxide		$12/g	PSR 1/2-20 μm; D = 8.3; P = 99.9
Platinum Metal		$12/g	PSR 1/2-40 μm; D = 21.4; P = 99.9
Palladium Metal		$12/g	PSR 1/2-30 μm; D = 12.0; P = 99.9
Platinum Sulphate		$12/g	----
Lead Chloride	J.T. Baker Chemicals Chicago	$6/lb	D = 5.85
Cadmium Oxide		$17/lb	D = 7.0
Manganese Dioxide		$6/lb	D = 5.0
Vanadium Pentoxide		$12/lb	D = 3.4
Manganous Oxide	M.C.B. Chemists Puratek Division Chicago	$1/g	P = 99.9; D = 5.4
Vanadium Trioxide		$0.3/g	P = 99.9; D = 4.9
Ferric Oxide	Charles Pfizer Reading, Pa.	---	PSR = 0.5-5 μm; D = 5.24; P = 99.8
Chromium Oxide		---	S.A. = 5 m^2/g; S = cylindrical
Nickel	Fisher Scientific Chicago	---	PSR 0.5-5.0 μm; D = 7.5 S.A. = 4 m^2/g; S = amorphous P = 99.8
Lead Oxide		---	P = 99.8; D = 9.5
Crude Air Particulate	E.P.A.	---	----

Key

Particle Size Range = PSR
Surface Area = S.A.
Shape = S
Purity = P
Density = D

where

d = Stoke's diameter in microns

h = height of fall = 10 cms

η = viscosity of IPA @ 25°C in poise

ρ_s = density of particles gm/cc

ρ_f = density of IPA @ 25°C in gms/cc

g = acceleration due to gravity cms/sec/sec

t = time in seconds

The powder was ultrasonically dispersed in a suspending fluid (usually isopropyl alcohol [IPA]), and introduced into the primary sedimentation flask, and made up to a volume at which the distance from the suspension surface to the discharge tap was known (usually equal to 10 cm). The suspension was well mixed and allowed to stand for time, t_1, calculated from Stoke's equation.

After time t_1, the primary vessel tap was opened and suspension containing particles of size less than d_1 was slowly run into vessel 2. Vessel 1 then contained particles of size greater than d_1 and the fines contained in the volume between the tap and the vessel base.

This was then refilled with clean fluid, mixed, and settled again. Meanwhile, vessel 2 was mixed and allowed to settle 10 cms for time t_2 which corresponded to a d_2 of 5 μm. After time t_2, the contents were run into vessel 3 where fractionation was performed to time t_3 which corresponded to a d_3 of 2 μm. As $t_3 > t_2 > t_1$ careful scheduling permitted the continuous fractionation and washing of all size fractions to be conducted on a daily basis, fines needing a longer time to precisely fractionate then coarse particles. Frequent examination of the fractions revealed that about 10 washings were required to produce powders to within each specification.

Toxicity and Reactivity of Powders

The potentially hazardous and toxic nature of the chemicals listed in Table 1 require special precautions during handling and use during fractionation, preparation, and characterization. For example, platinum or platinum oxide (PtO_2) in powdered form and isopropyl alsohol together under certain

conditions were found to be highly reactive. To minimize this hazard, double distilled water was used as the settling fluid for this powder/fluid combination.

Powder Characterization

All prepared powders were characterized as to particle size, surface area, particle shape, and purity. Particle size distribution was determined by sedimentation (Andreasen Pipet Method) or by optical microscopy. Surface area measurements were determined by use of the multi-point B.E.T. method. Particle shape was determined with the scanning electron microscope (SEM). Purity was determined by semi-quantitative arc emission spectroscopy to detect contaminants.

PREPARATIONS FOR STATE UNIVERSITY OF NEW YORK AT STONY BROOK (SUNY-STONY BROOK), SERIES I

Particulate preparations and suspensions were made for delivery to Dr. Philip Kane at SUNY-Stony Brook. Characterized platinum oxide (PtO_2), iron oxide (Fe_2O_3), and lead oxide (PbO) powder, both uncoated and coated with benzo(a)pyrene (1:1 weight ratio) were prepared and shipped once each three weeks. These were shipped dry. Suspensions of finely divided benzo(a)pyrene (1.25% by weight) in gel-saline along with gel-saline control samples were also prepared and shipped once each three weeks. The suspensions were placed in separate vials of approximately 20 ml per vial to approximate usage rate and thus minimize contamination from repeated opening of stock supply.

Benzo(a)pyrene applied as a coating to metal oxide particles has a tendency to crystallize into needles. Since this was undesirable in biological experiments, close coordination was required between the production of the coated powder and its time of use. As a precaution against delays in transit, the carrier's shipping number for each shipment was relayed to the recipient by telephone. With this information, a telephone search could be initiated immediately if the shipment did not arrive when expected.

Table 2 lists the various preparations delivered during this series to SUNY-Stony Brook.

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TABLE 2. PREPARATIONS FOR SUNY-STONY BROOK, SERIES I

Schedule	B.P.-Coated (1:1 wt. ratio)		Uncoated		One Week Ball Milled Suspension 0.5% Gel in 100 ml Saline Solution	
Date Shipped	Metal Oxide	Quantity, grams	Metal Oxide	Quantity, grams	1.25% B.P.	Control-No. B.P.
1) 01-06-75	Fe_2O_3	0.9	Fe_2O_3	0.4		
	PtO_2	0.8	PtO_2	0.4		
	PbO	1.0	PbO	0.4		
2) 01-13-75	Fe_2O_3	0.8	--	--	60 ml	60 ml
	PtO_2	0.6	--	--		
	PbO	0.9	--	--		
3) 01-28-75	Fe_2O_3	1.9	--	--		
	PtO_2	1.3	--	--		
	PbO	1.8	--	--		
4) 02-04-75	--	--	Fe_2O_3	1.2	60 ml	60 ml
	--	--	PtO_2	1.2		
	--	--	PbO	1.2		
5) 02-17-75	Fe_2O_3	1.9	Fe_2O_3	15.0		
	PtO_2	1.3	PtO_2	1.2		
	PbO	2.0	PbO	1.2		
6) 02-25-75	--	--	--	--	80 ml	80 ml
7) 03-10-75	Fe_2O_3	1.8	Fe_2O_3	1.2		
	PtO_2	1.3	PtO_2	1.2		
	PbO	1.7	PbO	1.2		
8) 03-11-75	--	--	--	--	80 ml	
9) 03-18-75	--	--	--	--	--	100 ml
10) 03-25-75	--	--	--	--	--	80 ml
11) 03-31-75	Fe_2O_3	1.8	Fe_2O_3	1.2	80 ml	--
	PtO_2	1.3	PtO_2	1.2		
	PbO	1.9	PbO	1.2		
12) 04-19-75	Fe_2O_3	1.8	Fe_2O_3	1.2	80 ml	80 ml
	PtO_2	1.3	PtO_2	1.2		
	PbO	1.7	PbO	1.2		
13) 05-19-75	Fe_2O_3	1.6	Fe_2O_3	1.2	80 ml	80 ml
	PtO_2	1.5	PtO_2	1.2		
	PbO	2.0	PbO	1.2		
14) 06-09-75	Fe_2O_3	1.8	Fe_2O_3	1.2	80 ml	80 ml
	PtO_2	1.3	PtO_2	1.2		
	PbO	1.4	PbO	1.2		
15) 07-01-75	Fe_2O_3	1.3	Fe_2O_3	1.2		
	PtO_2	1.8	--	--		
	PbO	1.5	PbO	1.2		
16) 07-21-75	--	--	--	--	80 ml	80 ml
17) 07-30-75	Fe_2O_3	1.6	Fe_2O_3	1.2	--	--
	PtO_2	1.3	PtO_2	1.2		
	PbO	1.5	PbO	1.2		
18) 07-31-75	--	--	--	--	--	80 ml
19) 08-20-75	Fe_2O_3	1.9	Fe_2O_3	1.2	100 ml	100 ml
	PtO_2	1.4	PtO_2	1.2		
20) 09-03-75	Fe_2O_3	1.8	Fe_2O_3	1.2	80 ml	80 ml
	PtO_2	1.5	PtO_2	1.2		
21) 09-18-75	PbO	2.1	PbO	1.2	-	--
	PbO	2.2	PbO	1.2		

PREPARATIONS FOR IITRI LIFE SCIENCES DIVISION, SERIES I

Biological experiments in IITRI's Life Sciences Division were supported by preparations of Platinum Oxide (PtO_2) and Palladium Oxide (PdO). These powders required size fractionation by the cascade sedimentation process.

Table 3 is a description of the delivered preparations:

TABLE 3. PREPARATIONS FOR IITRI LIFE SCIENCES, SERIES I

Date Shipped	Metal Oxide	Size Range	Quantity Shipped
01-04-75	PtO_2	1-10 μm	55.4 gm
03-10-75	PtO_2	1-10 μm	72.2 gm
03-23-75	PdO	1-10 μm	55.5 gm

POWDER PREPARATIONS FOR EPA/CINCINNATI

Preparation and characterization of submicron Pt/Pd-coated alumina automotive catalytic converter dust were made for Dr. Wellington Moore at EPA/Cincinnati. The objective of the work was to produce 1 kg of powder not exceeding 1 μm in particle size. A combination of grinding and size fractionation was to be used to achieve this size. Physical and chemical characteristics pertinent to the powder were to be obtained.

The Pt/Pd-coated Al_2O_3 catalyst powder was ground by wet ball milling for two weeks in a 14 cm diameter porcelain milling vessel using 1 cm diameter porcelain balls. The ground material was check-d for size by microscopic examination.

In addition to microscopic examination, the size of the parent and ground Al_2O_3 powder was determined by means of a Micromeritics Model 5000 Sedigraph particle size analyzer. In a preliminary step, the density of the powder was determined to be 3.04 g/cc by means of a liquid pycnometer. The powder was dispersed in water (2 g/30 ml) using a Waring blender and 0.1% tetrasodium pyrophosphate (TSPP) surfactant.

The parent powder was too coarse for direct analysis by the Sedigraph. Therefore, a preliminary wet sieving step was done using a 200 mesh (74 μm) screen. It was found that 17.3% by weight was retained on the screen. The fraction passing the screen was then run on the Sedigraph. The combined size distribution is shown in Figure 1.

Figure 1 shows that the mass medium diameter of the parent material is 2.0 μm (equivalent spherical diameter). By comparison, the ground material has 90% by mass below 1.0 μm, and 80% by mass is less than 0.3 μm. The mass median diameter of the ground material is probably below 0.1 μm.

Surface area measurements for the ground Al_2O_3 were run by two methods -- the Micromeritics Model 2200 and Quantachrome "Monosorb" device. The results were:

by Micromeritics Model 2200 -- 92.1 m^2/g

by Quantichrome "Monosorb" -- 77.3 m^2/g

The two values agree reasonably well. The former value is probably more nearly correct, since the latter value was obtained during an apparatus demonstration.

A pore volume analysis was obtained by mercury porosimeter. The total pore volume was found to be 1.12 cc/g. One half of this volume was attributed to pores smaller than 0.13 μm.

The trace chemical analysis on the parent and ground Al_2O_3 was done by arc-induced emission spectroscopy. The results are shown in Table 4. It is seen that the grinding process did not alter the concentrations of the catalyst materials, palladium, and platinum. However, there was an enhancement of the silicon, magnesium, and titanium content, probably due to erosion of the mill parts.

As a further chemical test of the parent and ground powders, x-ray emission spectra were obtained with a scanning electron microscope. In this test, the analyst could not detect platinum or palladium on either the parent or ground Al_2O_3. Apparently, the catalyst materials are embedded in pores not accessible to the electron beam used to exite the x-rays. The analyst did

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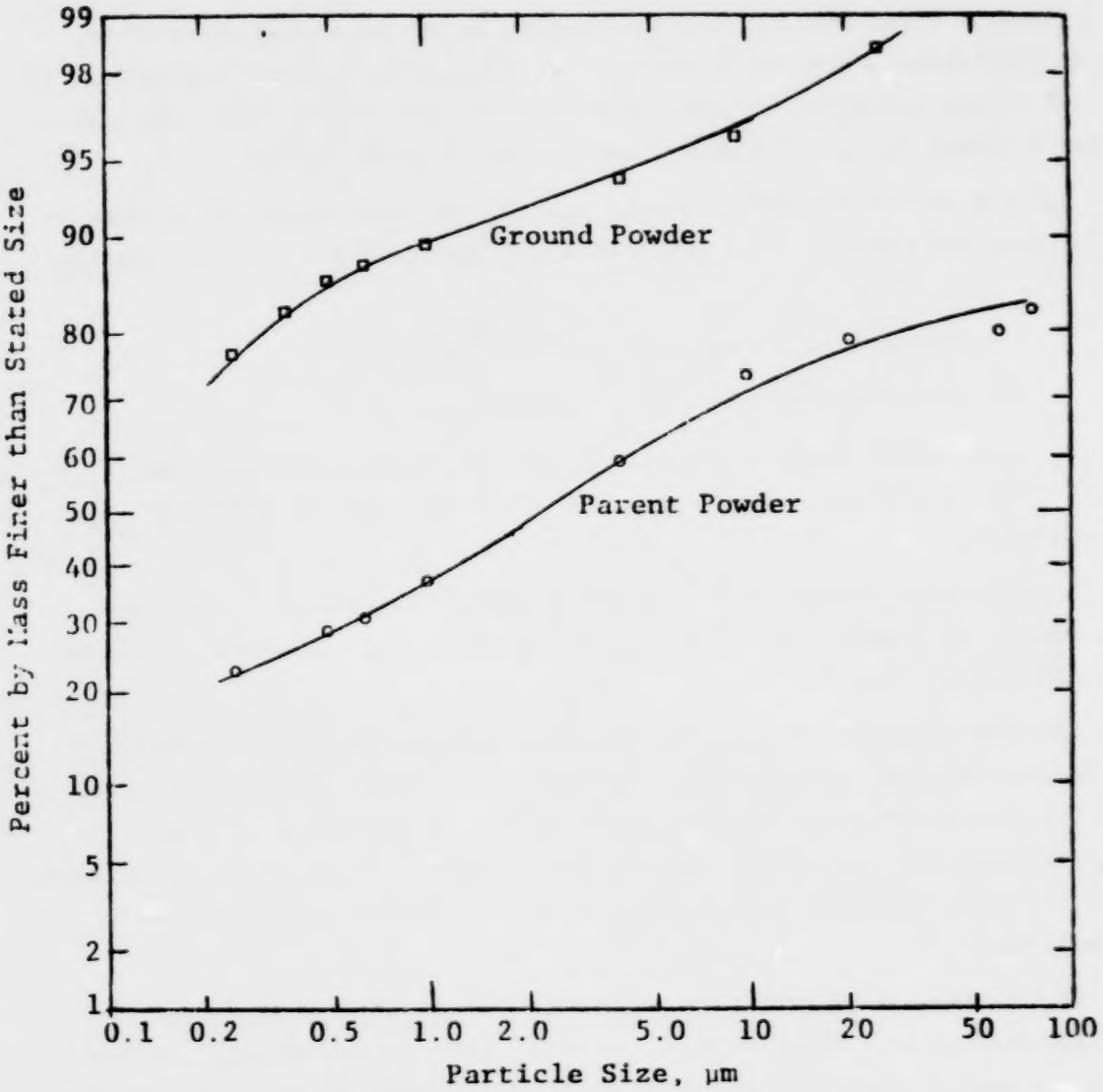

Figure 1. Size distribution of parent and ground catalyst powder.

TABLE 4. TRACE CHEMICAL ANALYSIS OF PARENT AND GROUND CATALYST POWDER*

Element	Weight Percent of Total	
	Parent	Ground
Si	0.1	0.4
Mg	0.1	0.2
Ca	0.02	0.02
Cu	0.002	0.002
Ti	0.002	0.01
Sr	0.001	0.001
Fe	0.1	0.1
Pt	0.01	0.01
Pd	0.02	0.02

* Arc-induced emission spectroscopy.

find traces of calcium in the parent material and traces of silicon in the ground material.

The arc-induced emission spectrograph yields a chemical analysis by elements which is only semi-quantitative. More accurate analysis for specific metals can be obtained by atomic absorption spectrophotometry, as was done for the parent powder by R.G. Miller of EPA's Environmental Toxicology Laboratory in Cincinnati. His results were: Pt, 0.042% by weight; Pd, 0.016% by weight.

PREPARATIONS FOR IITRI LIFE SCIENCES DIVISION, SERIES II

Another series of biological experiments conducted by IITRI's Life Sciences Division under EPA contract required preparations of fractionated fly ash material both uncoated and coated with a selected metal oxide. The effect of particle size as well as the effect of the particle's metal oxide coating on phagocytosis of fly ash particles by rabbit lung cells were being investigated.

The size ranges of fly ash required were: 0-2 μm, 2-5 μm, and 5-10 μm. The required coating materials were the oxides of nickel, manganese, lead, cadmium, and vanadium. A stock of fly ash material was supplied by Dr. Donald Gardner at EPA/RTP.

Size classification of fly ash is not straightforward due to the heterogeneous nature of the material. The particles range from glassy silicate spheres, some of which are hollow, to hematite particles. Accordingly, the density varies considerably from particle to particle. Some particles have a specific gravity less than unity. Air classification using a Bahco Microparticle Classifier (Harry Dietert Co., 9220 Roselawn Ave., Detroit, Michigan) was adopted. This technique is sanctioned by the American Society of Mechanical Engineers in the ASME Powder Test Code No. 28 as the recommended method for determining the size distribution of fly ash (1). The results are shown in Table 5.

The Bahco Microparticle Classifier has size cut points which closely match those desired for the fly ash fractions. Furthermore, the method is an accepted standard for fly ash fractionation. For these reasons, the fly ash fractions delivered to IITRI Life Sciences were obtained using the Bahco. The size ranges were:

TABLE 5. SIZE DISTRIBUTION OF PARENT FLY ASH

Size Range*, um	Percent of Total Mass in Size Range
0-2.2	7.0
2.2-5.0	35.0
5.0-8.0	14.5
over 8.0	43.3

TABLE 6. WEIGHT PERCENT CONCENTRATION OF SELECTED ELEMENTS IN VARIOUS FLY ASH SAMPLES

Element	Present Fly Ash, Emission Spectroscopy	NBS Fly Ash Standard, SRM #1633			Lee, et al (3)
		ORNL	NBS	Ondov	
Fe	10.	6.37			15.4
Si	10.			21	
Mg	1.		1.98	1.8	
Al	2.	12.5		12.7	
V	0.5	0.024	0.0214	0.0235	0.017
Ca	1.	4.34		4.7	
Cu	1.	0.0133	0.0120		
Ti	1.	0.642		0.740	
Ni	0.01	0.0109	0.0098	0.0098	0.015
Mn	0.05	0.046	0.0493	0.0486	
Sr	0.1	0.1201	0.1380	0.1700	
Cr	0.05	0.0138	0.0130	0.0127	0.008
Ba	0.05	0.278		0.270	

* Result of Oak Ridge National Laboratory, National of Standards and Ondov, et al., Analyses, reported in Reference 4.

Fine fraction -- 0-2.2 μm

Intermediate -- 2.2-5.0 μm

Coarse fraction -- 5.0-8.0 μm

It is well to point out that the particle classification obtained by the Bahco are according to aerodynamic equivalent diameter. These differ somewhat from the geometric diameters as determined by microscope. A large, hollow particle, for example, behaves aerodynamically like a smaller, solid particle, and is so classified by the Bahco. Hence, photomicrographs may show a number of large, hollow particles in the fine size fraction.

A sample of the parent fly ash was screened by arc-induced emission spectroscopy to determine the elemental composition. The results are shown in Table 6. These results are semi-quantitative only, and were done mainly to provide a base line against which to compare the coated materials.

Also shown in Table 6 are results of elemental analysis of various fly ash samples reported in the recent literature (2,3,4,5,6). The National Bureau of Standards fly ash standard (Standard Reference Material No. 1633) is particularly well studied. These results are shown for comparison only, and they indicate that substantial differences in composition can exist between different lots of fly ash.

References 2 and 3 show that the elemental composition of fly ash varies with particle size and with point of collection (before or after the emission control device). Therefore, different size fractions of fly ash will have different elemental composition. This should be kept in mind when interpreting the results of biological experiments involving fractionated fly ash.

The technique devised for coating fly ash with oxides of nickel, lead, or manganese was patterned after that described by Pupynin, et al. (7). In brief, the technique was as follows: dissolve 0.3 g of the chloride salt of the desired metal in 200 ml of water; chill the solution to 3°C; add 3 g of the desired fly ash fraction while stirring continuously; add the equivalent weight of sodium hydroxide, one drop at a time, to precipitate the metal as fine metal hydroxide; filter the suspension using a Buchner funnel; wash to remove soluble sodium salts, dry the filter cake in a muffle furnace at 600°C

to cause conversion to metal oxide. The procedure is outlined more completely in Appendix A.

Microscope examination of the dried material after crushing the filter cake revealed a degree of coating which varied with the metal. Lead oxide produced the best coating of the four tried. The lead oxide coatings were of a quality similar to our benzo(a)pyrene-coated particles. Vanadium oxide coating of fly ash was not possible. The solubility of the oxide prevented the preparation of a suitable product for biological use. All attempts to prepare a precipitate free of soluble vanadium compounds failed.

An assay of the metal content of the final coated samples delivered to IITRI Life Sciences was obtained by atomic absorption spectrophotometry. Approximately 10 mg of each coated fly ash was weighed accurately and digested in concentrated nitric plus hydrochloric acid. These samples were then brought to 10 ml total volume by adding deionized water. Similarly, approximately 25 mg of each uncoated fly ash fraction was weighed, digested, and brought to 25 ml total volume. Standard solutions were also prepared for each metal, and all standards and samples were analyzed by direct aspiration atomic absorption spectrophotometry. The results are shown in Table 7.

During the IITRI Life Sciences experiments with the fly ash fractions, a question came up concerning the number of particles per unit mass for each of the fly ash samples. Therefore, a technique based on microscopy was devised to obtain this data. This technique is described in detail in Appendix B.

The results of these counts are shown in Table 8. It is noted that in the small size fraction, the application of the coatings substantially decreased the number of particles per unit mass. With one exception, this decrease did not occur for the two larger size fractions. The microscopic observations indicated that the decreased number in the coated samples for the small size fraction was due to aggregates of up to about six fly ash particles. The uncoated material was well dispersed as single particles. The particle size range for the small size fraction, as determined by microscope, was 0.5 to 4.0 μm.

TABLE 7. ATOMIC ABSORPTION ANALYSIS OF FLY ASH FRACTIONS

Size Fraction	Element	Concentration of Metal in Weight Percent				
		Uncoated Fly Ash	Lead Coated Fly Ash	Nickel-Coated Fly Ash	Manganese-Coated Fly Ash	Cadmium Coated Fly Ash
0.0 - 2.2 μm	Pb	<.03	3.85			
	Ni	<.03	--	2.63	--	--
	Mn	<.01	--	--	3.22	--
	Cd	<.02	--	--	--	12.05
2.2 - 5.0 μm	Pb	<.03	3.84	--	--	--
	Ni	<.03	--	2.91	--	--
	Mn	<.01	--	--	3.67	--
	Cd	<.02	--	--	--	11.91
5.0 - 8.0 μm	Pb	<.03	3.15	--	--	--
	Ni	<.03	--	2.77	--	
	Mn	<.01	--	--	2.91	--
	Cd	<.02	--	--	--	12.30

TABLE 8. NUMBER OF FLY ASH PARTICLES PER UNIT MASS OF FLY ASH

Nominal Size Fraction	Number of Particles per mg of Fly Ash				
	Uncoated Fly Ash	PbO-Coated Fly Ash	NiO-Coated Fly Ash	MnO_2-Coated Fly Ash	CdO-Coated Fly ASh
0.0 - 2.2 μm	2.3×10^8	1.05×10^8	3.8×10^7	5.8×10^7	3.2×10^7
2.2 - 5.0 μm	2.3×10^7	4.6×10^7	2.2×10^7	2.6×10^7	2.8×10^6
5.0 - 8.0 μm	1.6×10^7	1.2×10^7	4.4×10^6	1.7×10^7	1.3×10^4

PREPARATIONS FOR SUNY-STONY BROOK, SERIES III

A second series of preparations for biological experiments was initiated for Dr. Philip Kane at SUNY-Stony Brook. A modified milling procedure was initiated to overcome some of the earlier uncertainties in preparatory technique. The essential change in technique for the BP/metal oxide suspensions was the addition of fractionated metal oxide particles with all other ingredients except the BP for sterilization in the autoclave prior to the "ball-milling" step. The intended benefits in the change of procedure were:

1. To minimize contamination.

2. To obtain a more uniform BP/metal oxide homogeneity and particle distribution.

To maintain continuity and a basis for comparison, all ingredients were from the original stock in the Series I preparations. The BP and platinum oxide (PtO_2) were provided by Dr. Kane. All glassware, equipment to be in contact with the suspension, the required quantity of metal oxide, gelatin, and saline solution were sterilized in the autoclave. The BP was then added to the autoclaved suspension prior to milling. Approximately 100 ml of milled suspension were required per preparation. Milling time was seven days. The milled suspension was then divided into four vials of approximately 20-23 ml of suspension per vial, the balance being retained for particle size analysis, BP assay, and sterility.

Data on these preparations are presented in Table 9. Table 10 presents results of the spectrochemical analysis for the contaminants present in the metal oxides used.

Initially the four vials (80-90 ml) were sealed, taped, and packed with canned ice in an insulated shipping container for shipment. Air freight and air mail special delivery as shipping methods were found to be deficient in attaining a precise delivery time table. Express mail service (guaranteed next day delivery) was found to be the fastest and most reliable shipping method. Each vial was individually bagged, then the group bagged into another plastic container, and the groups bagged into a larger leak-proof container. These bags within a bag were then packed snugly in an insulated shipping container.

TABLE 9. PREPARATIONS FOR SUNY-STONY BROOK, SERIES III

Date	Preparation	Particle (Number % Less than Stated Size)						Assay, %	
		0.75μ	1.5μ	3.0μ	6.0μ	7.3μ	12.0μ	B.P. (Spectrophotometer)	Metal Oxide (Gravimetric)
05-10-76	IA 2% Suspension, 1:1 BP/Fe_2O_3	13.4	24.8	50.2	77.1	91.5	100.0	2.00	2.03
	IB 2% Suspension, 1:1 BP/PtO_2	8.1	23.1	54.3	88.2	95.2	100.0	2.10	2.00
	IC 2% Suspension, 1:1 BP/PbO	56.8	74.0	89.9	97.0	99.6	100.0	2.10	2.16
06-10-76	IIA 2% Suspension, 1:1 BP/Fe_2O_3	14.2	25.6	50.3	77.2	91.4	100.0	2.12	2.23
	IIB 2% Suspension, 1:1 BP/PtO_2	10.7	24.3	54.9	85.5	93.7	100.0	2.12	2.31
	IIC 2% Suspension, 1:1 BP/PbO	54.9	72.8	88.1	94.9	97.5	100.0	2.12	2.02
07-06-76	IIIA 2% Suspension, 1:1 BP/Fe_2O_3	--	--	--	--	--	--	CONTAMINATED	
	IIIB 2% Suspension, 1:1 BP/PtO_2	12.8	36.1	68.4	88.9	96.9	100.0	2.12	2.00
	IIIC 2% Suspension, 1:1 BP/PbO	54.1	73.0	89.2	96.9	98.8	100.0	2.12	2.00
07-20-76	IIIA-1 2% Suspension, 1:1 BP/Fe_2O_3	--	--	--	--	--	--	CONTAMINATED	
07-29-76	IIIA-2 2% Suspension, 1:1 BP/Fe_2O_3	--	--	--	--	--	--	CONTAMINATED	
08-02-76	IV A 2% Suspension, 1:1 BP/Fe_2O_3	12.7	26.2	50.1	72.1	86.8	100.0	2.00	2.63
	IV B 2% Suspension, 1:1 BP/PtO_2	10.2	26.7	58.0	88.0	95.5	100.0	2.00	2.00
	IV C 2% Suspension, 1:1 BP/PbO	54.1	72.9	90.7	96.9	99.1	100.0	2.00	2.00
08-09-76	IIIA-3 2% Suspension, 1:1 BP/Fe_2O_3	--	--	--	--	--	--	CONTAMINATED IN USE	
	IIIA-4 2% Suspension, 1:1 BP/Fe_2O_3	13.8	25.2	50.2	77.1	91.4	100.0	2.10	2.30
08-26-76	V A 1.5% Suspension, 1:1 BP/Fe_2O_3	--	--	--	--	--	--	CONTAMINATED	
	V B 1.5% Suspension, 1:1 BP/PtO_2	8.3	30.1	59.4	80.7	90.5	100.0	1.51	1.54
	V C 1.5% Suspension, 1:1 BP/PbO	48.4	70.3	87.0	95.8	98.9	100.0	1.50	1.51
09-01-76	V A-1 1.5% Suspension, 1:1 BP/Fe_2O_3	5.2	21.2	47.9	76.3	91.8	100.0	1.50	1.50
09-21-76	VI A 1.5% Suspension, 1:1 BP/Fe_2O_3	21.2	28.1	52.7	78.3	91.6	100.0	1.50	1.50
	VI B 1.5% Suspension, 1:1 BP/PtO_2	7.7	16.5	50.0	95.4	100.0	--	1.50	1.50
	VI C 1.5% Suspension, 1:1 BP/PbO	63.1	76.9	92.3	98.1	100.0	--	1.50	1.50

TABLE 10. TRACE CHEMICAL ANALYSIS OF METAL OXIDES

Fe_2O_3		PbO		PtO_2	
Constituent	Concentration, % (Estimate)	Constituent	Concentration, % (Estimate)	Constituent	Concentration, % (Estimate)
Fe	Major	Pb	Major	Pt	Major
Mn,Zn	0.05-0.5	Bi	0.01-0.1	Zn	0.05-0.5
Ni,Mg,Ca	0.005-0.5	Si	0.005-0.05	Ni	0.03-0.3
Si,Al	0.003-0.03	Ca	0.001-0.01	Cu,Pd,Ca,Fe	0.005-0.05
Cr,Mo,Co	0.001-0.01	Fe	0.0003-0.003	Si,Pb,Au,Ag	0.002-0.02
Cu,Pb	0.0005-0.005	Al,Mg,Cu,Ag	0.0001-0.001	Ir,Mg,Ru,C_L	0.001-0.01
				Al	0.0005-0.005
				Mn	0.0001-0.001

SECTION 3

RESULTS AND CONCLUSIONS

Series II in the preparations for SUNY-Stony Brook is still continuing. Negotiations are in progress for continued funding for this as well as other preparation and characterization work on particulate contaminants for biological experiment.

APPENDIX A

PROCEDURE FOR COATING FLY ASH WITH NICKEL, MANGANESE, OR LEAD OXIDES

1. Use the Bahco air classifier to separate the fly ash into three size ranges, each weighing 3 grams: (0-2.2) μm, (2.2-5.0) μm, and (5.0-8.0) μm.

2. Dissolve 0.300 g of a chloride salt in 200 ml H_2O from one of the above metals for each size fraction to be coated.

3. Pour in one 3 g size fraction of fly ash and cool mixture to 3°C. Stir mixture continuously.

4. Add equivalent weight of NaOH (drop at a time) to cold stirred mixture and continue stirring for one hour.

5. Filter the metal hydroxide coated fly ash using a Buchner funnel.

6. Dry the coated material in a muffle furnace at 657°C. This temperature will convert most of the hydroxide to the oxide.

7. Assay the dried sample to determine amount of metal (Ni, Mn, Cd, or Pb) present.

APPENDIX B

NUMBER OF PARTICLES PER UNIT MASS FOR THE COATED AND UNCOATED FLY ASH FRACTIONS

The technique for determining the number of particles per unit mass of fly ash was as follows: a 3 to 5 mg amount of the fly ash sample was weighed to the nearest 0.1 mg and suspended in 20 ml of growth medium 199. This was shaken vigorously and treated for 15 seconds in an ultrasonic bath to disperse the fly ash. The suspending medium, the concentration, and the dispersing technique was selected to resemble those used in the IITRI Life Sciences Division experiments.

Next, a second suspension was made up consisting of paper mulberry pollen suspended in distilled water at a concentration of about 1.0 mg/ml. After vigorous shaking, a drop of the pollen suspension was mounted on a hemacytometer, and the number of particles per ml of suspension was determined. The large (16 μm diameter) uniformly sized pollen particles served as an internal standard in the later counting of fly ash particles.

A third suspension was then prepared by mixing equal volumes of the fly ash suspension and the paper mulberry pollen suspension. A drop of this mixed suspension was placed on a microscope slide and covered with a cover slip. After an initial inspection at low magnification to insure that the particles were dispersed and that the sample was uniformly spread on the slide, counts were made of both the fly ash and the pollen particles. The fly ash particles were counted in several view-fields at 500X, and the pollen particles were counted in several view-fields at 125X. The 500X magnification used for fly ash made it possible to count particles as small as 0.5 μm.

From the above data, the number, N, of fly ash particles per mg of fly ash was determined from the equation

$$N = \frac{n_{fa}}{n_p} \cdot \frac{s_p}{m_{fa}}$$

where

n_{fa} = the number of fly ash particles per unit view-area (from the count on the mixed suspension).

n_p = same as above for the pollen particles.

s_p = the number of pollen particles per ml of suspension 2 (from the hemacytometer count).

m_{fa} = mg of fly ash per ml of suspension 1.

(A factor was applied to the number of fly ash and pollen particles counted to place these numbers on an equal view-area basis.)

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